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## **A survey of bovine viral diarrhea virus testing in diagnostic laboratories in the United States from 2004 to 2005**

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**Abstract.** Bovine viral diarrhea virus (BVDV) has a great economic impact on the United States cattle industry. The Academy of Veterinary Consultants, the American Association of Bovine Practitioners, and the National Cattlemen's Beef Association have called for the goal of BVDV control and eventual eradication in the USA. One of the key factors in such efforts will be the detection of BVDV infections, particularly targeting persistently infected animals. To assess current BVDV detection methods in the USA, 26 veterinary diagnostic laboratories in 23 states were surveyed. Survey questions related to the types of tests currently offered, the number of tests performed, the reasons for test requests, the type of samples used, whether sample pooling was performed, and whether follow-up testing or information regarding bovine viral diarrhea (BVD) management was provided after positive tests. There was no clear consensus on an individual BVDV testing method, the pooling of samples or the retesting of positive animals. Ear-notch antigen capture enzyme-linked immunosorbent assay (ACE) was the test most frequently performed based on the absolute number of

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tests. However, when the data were adjusted to reflect individual laboratory choices, the number of ACE and immunohistochemistry tests performed on ear notches was nearly equal. Only 55% of diagnostic laboratories provided BVD management information to producers or veterinarians who submitted positive samples. There was no significant difference in the number of positive tests in laboratories that received the majority of their samples for screening purposes versus laboratories that received the majority of their samples because BVDV was suspected based on clinical signs in a herd.

**Key words:** Bovine viral diarrhea virus; control; diagnostic tests; eradication; survey.

Bovine viral diarrhea virus (BVDV) associated diseases cause a wide range of clinical presentations in cattle. Acute infections are frequently subclinical in nature but can result in respiratory disease, gastrointestinal disease, and immunosuppression, contributing significantly to production loss. In breeding herds, BVDV infections cause abortions, stillbirths, and congenital defects. Bovine viral diarrhea virus infection in pregnant animals can also result in the birth of a persistently infected (PI) calf. Some highly virulent strains of BVDV cause severe disease, including hemorrhagic syndrome. Persistently infected cattle may succumb to a highly fatal form of BVD called mucosal disease. The combined economic impact of BVDV has been estimated at a 20 to 57 million dollar loss per million calvings in the USA.<sup>6</sup> Based on the 2005 US calf crop of 38 million, the cost of BVDV to US producers was 760 million to 2.2 billion dollars. The economic impact of BVDV led the Academy of Veterinary Consultants, the American Association of Bovine Practitioners, and the National Cattlemen's Beef Association to adopt resolutions calling for the reduction and the eventual eradication of BVDV.

In contrast to other acute viral infections, BVDV control focuses on the identification of PI animals, because PI cattle serve as the main source of BVDV infection because of the large amount of virus shed.<sup>8,10,16</sup> An epidemiologic study showed that cattle exposed to PI animals had a 48% greater incidence of bovine respiratory disease (BRD) and increased risk of treatment for BRD, as well as an increased number of treatments compared with cattle not exposed to PI animals.<sup>11</sup> Current practices in the cattle industry do not limit the risk of exposure to PI animals. A recent feedlot study showed that 30.8% of 240 pens would contain at least 1 PI animal, given a prevalence of 0.4% PI animals and a total of 21,743 head.<sup>4</sup>

Information regarding current BVDV testing practices would be helpful as a unified approach to BVDV control is developed. The goal of this study was to survey current BVDV testing practices in diagnostic laboratories to determine the types of tests used, the application of tests, and the actions taken when positive tests occur. The information gathered from this survey is important because the choices of BVDV testing in diagnostic laboratories would have a direct impact on the control of BVDV.

The current array of diagnostic tests available is large, and the application and the proficiency of testing is variable. The ideal BVDV diagnostic test should be inexpensive, quick to perform, have high sensitivity and specificity, and should use samples that are easy to collect.<sup>1,4</sup> There is currently no test that meets all of these criteria in all situations, and diagnostic laboratory custo-

mers must select from the array of test characteristics to choose what best fits their situation without the benefit of definitive guidelines to help them make that selection. The ramifications of their testing choice can impact BVDV disease control. In addition, the range of clinical presentations and the severity of BVDV infections makes detection of BVDV infected herds difficult based on clinical signs. Testing herds for BVDV solely based on clinical signs may result in missed diagnoses and the perpetuation of the virus infection. To investigate current BVD diagnostic methods, a survey was sent, in an electronic format, to 46 diagnostic laboratories identified as accredited laboratories by the American Association of Veterinary Laboratory Diagnosticians (AAVLD).

The electronic survey consisted of 10 questions covering several broad topics of how diagnostic laboratories approached BVDV testing (Appendix 1). The respondents were instructed to report data acquired in a 12-month period beginning October 1, 2004, and ending October 1, 2005. Virus isolation (VI); reverse transcription-polymerase chain reaction (RT-PCR) on serum, buffy coat, or milk; ear-notch immunohistochemistry (IHC); and antigen capture antigen capture enzyme-linked immunosorbent assay (ACE) on serum or ear-notch extracts were included in the panel of tests listed. A space to fill in tests other than those listed was provided. A percentage was calculated for the usage of each test type by using the reported total number of tests performed in each test category for all laboratories divided by the total number of tests performed overall. Additional calculations were performed by deriving a percentage usage for each test type per laboratory and summing these percentages per test type, then recalculating a ratio. This calculation will be further referred to as an adjustment to reflect individual laboratories, because it removes the bias that larger laboratories have toward their preferred testing type because of the large number of tests performed. Antibody-detection-based tests were not included in the list of test types because they are unreliable in identifying PI animals and herd exposure to natural infection as determined by serology can be compromised by vaccination.<sup>5</sup>

Diagnostic laboratories were asked to report the total number of BVD tests and positive tests for the given time period. The literature suggests that a follow-up test should be performed 3 weeks later for positive animals to determine if they are truly PI or if they were acutely infected at the time of initial sampling.<sup>1,5,8</sup> Therefore, diagnostic laboratories were asked if they recommended a second test to be performed for all BVDV-positive tests. Diagnostic laboratories were also asked to report whether they provided any follow-up education/counseling to veterinar-

ians and/or producers who had positive test results. The cost of BVDV testing can affect use and is a practical factor in diagnosis. To defray cost, pooling of samples has been considered for some of the available tests. Therefore, diagnostic laboratories were asked if they pooled samples and what pool sizes they used.

BVDV control and eradication efforts in other countries use herd screening.<sup>5,9-10,12</sup> The current level of BVDV screening in the USA is useful information for BVDV control, therefore, diagnostic laboratories were asked to estimate the percent of samples that were submitted for BVDV herd screening purposes and the percent of samples that were submitted because the animal was a BVDV suspect because of clinical signs. In addition, the correlation of the number of positive tests with the reason for sample submission can be used to address the concern that BVDV-infected animals are difficult to pinpoint because of the potential subclinical nature of BVDV and the wide range of clinical signs that may occur. A *t*-test was used to compare the percent positive tests between these 2 groups.

Responses were received from 26 diagnostic laboratories, representing 23 different states. One laboratory provided data for the fiscal 2004 year, which was out of the parameters for dates requested. However, these data were deemed relevant to the study and, therefore, were included. Twenty laboratories returned completed surveys; 6 laboratories returned partially completed surveys. Data were used from partially completed surveys when an independent series of questions were complete. Most laboratories (77% of 26 laboratories) offered a variety (4 to 7 types) of testing methods, with 15% of 26 laboratories offering 1 to 3 testing types, and 8% of 26 laboratories offering 8 or more testing types. There was no strongly favored testing method offered among the responding laboratories. The percentage of laboratories offering each test was as follows; 85% of laboratories (*n* = 26) offered VI; 73% of laboratories (*n* = 26) offered a form of PCR with 50% offering PCR on serum, 63% offering PCR on buffy coat, and 29% offering PCR on milk; 69% of laboratories (*n* = 26) offered serum ACE; 65% of laboratories (*n* = 26) offered ear-notch ACE; and 88% of laboratories (*n* = 26) offered a form of IHC with 69% offering ear-notch IHC. Ten laboratories elected to report the use of BVDV fluorescent antibody (FA) and 4 laboratories elected to report the use of BVDV virus neutralization (VN) tests. These test categories were considered in the analysis of the data on an individual laboratory basis but were not included in group data. For example, the VN tests were counted as an additional test type offered by a laboratory, and the numbers reported for that test category were used when counting total number of tests for the individual laboratory.

All laboratories offered individual sample testing, whereas 46% of laboratories (*n* = 24) offered testing of pooled samples. The 11 laboratories that offered sample pooling had highly variable upper limits for the number of samples to be pooled; the mean upper limit was 40 samples, the median was 14 samples, and the range was 2 to 120 samples. Some laboratories specified different upper limits for different test types, e.g., 10 animals for RT-PCR on serum and 120 animals for RT-PCR in bulk milk samples.

The total number of BVDV tests that were performed during the 1-year period by the 26 reporting laboratories was 445,648. There was a wide range of sample numbers processed by the responding laboratories; the mean number of BVDV tests run per laboratory during the reporting period was 19,376, the median was 7,482, and the range was 90 to 59,728. Virus isolation was 6.5% of the total number of tests run and was 6.7% when the data were adjusted to reflect individual laboratories. Polymerase chain reaction was 2.2% of the total number of tests run and was 4.5% when the data were adjusted to reflect individual laboratories. Ear-notch ACE tests were run the most and accounted for 44% of the total number of tests. Ear-notch IHC was 14% of the total number of tests run. However, when the data were adjusted on a per laboratory basis, ACE on ear notches (24% of all tests with *n* = 22 laboratories), and ear-notch IHC (25% of all tests with *n* = 22 laboratories) were chosen in nearly equal numbers. Serum ACE was 13% of the total number of tests run and was 13% when the data were adjusted to reflect individual laboratories.

The mean number of positive tests was 4.3%, the median was 1.1%, and the range was 0.3% to 26.1% (*n* = 23 laboratories). The number of positive tests reported for each laboratory is the total number of positive tests and not necessarily the total number of positive animals. Laboratories were asked a follow-up question as to whether the reported total positive tests included repeat positive tests on the same animal. Eight laboratories responded to this question: 5 stated that the number of positive of tests reported represented all single tests, and 3 reported that positive tests included a proportion of follow-up tests. When regarding laboratory actions after a positive test, 55% of laboratories (*n* = 22 laboratories) offered written and/or oral follow-up information to those who submitted positive tests, and 61% of laboratories (*n* = 23) recommended retesting positive animals.

When queried as to the major reason samples were submitted for testing, the 22 responding laboratories were split 50%/45% (5% of laboratories had no one prevailing reason for sample submission) between the majority of samples being submitted for screening purposes (75%–95% of the requested BVDV tests) and the majority of samples submitted because of clinical presentation suggestive of BVD (70%–100% of the requested BVDV tests). The percentage of positive tests found by those laboratories receiving the majority of their samples as the result of BVDV screening efforts was 1% (mean) and 0.6% (median). The percentage of positive tests in those laboratories that received the majority of their BVDV test requests as the result of clinical presentations suggestive of BVD was 6.6% (mean) and 1.3% (median). There was no significant difference at a 99% confidence interval of the number of positive tests between laboratories that received the majority of their BVDV samples for screening purposes versus those that received the majority of their BVDV samples from suspected BVD cases based on clinical signs.

The survey respondents consisted of a representative sample of diagnostic laboratories across the USA and

included 63% of the diagnostic laboratories accredited by the AAVID. The results of this survey indicate that there is no clear consensus on a standard method for BVDV testing or for types of BVDV tests offered, and there is no standard policy on pooling samples for tests or an upper limit of samples in each pool. Currently, most diagnostic laboratories are offering an array of BVDV test types, most likely because no one test is considered perfect in all situations.

Virus isolation using cultured bovine cells and blood or tissue samples is considered the gold standard for BVDV detection. However, VI is labor intensive and time consuming.<sup>1</sup> Animals with positive tests must be retested 3 weeks later to confirm their PI status because VI may also detect acutely infected cattle.<sup>1,8</sup> A study that compared VI and RT-PCR revealed that 19% of 16 PI animals were missed by VI;<sup>2</sup> whereas, in another study, VI results gave the best correlation with PI status.<sup>1</sup> In addition, there are multiple protocols for BVDV VI procedures, which may explain inconsistencies in VI results between laboratories.

Reverse transcription-PCR can detect BVDV RNA in buffy coat cells, whole blood, serum, semen, and ear-notch samples. This technique is highly sensitive and is frequently used on pooled samples to reduce the expense of testing.<sup>5,16</sup> However, RT-PCR is a time consuming, multiple-step process that increases the risk for contamination, and primers must be carefully selected to detect current BVDV strains.<sup>16</sup> Repeat testing of RT-PCR positive animals is required to establish persistent infection because the sensitivity of the technique. In 1 study,<sup>15</sup> 19% of 16 acutely infected cattle were positive via RT-PCR.

Ear-notch IHC has been shown to be accurate and effective in detecting PI animals; 2 recent studies showed the technique identified 100% of PI animals that were also identified by VI and RT-PCR.<sup>1,4</sup> Samples for ear-notch IHC have been shown to be stable; they can be stored in formalin for up to 30 days or in a refrigerator for up to 10 days before processing without causing a false-negative IHC test.<sup>13</sup> Disadvantages of the IHC test are that the test is labor intensive, with multiple steps that increase the risk for processing errors and that has subjective evaluation criteria.<sup>1</sup> Conflicting information regarding the ability of the IHC to detect acutely infected animals has been published. In a 2002 study,<sup>15</sup> none of 16 acutely infected cattle were positive on ear-notch IHC. However, a recent study<sup>1</sup> found that 3 of 8 acutely infected cattle were positive on ear-notch IHC for up to 8 months.

Antigen capture enzyme-linked immunosorbent assay testing can be performed on serum samples or extracts of ear notches in PBS. One of the benefits of ACE is that tests can be completed within hours.<sup>1,4</sup> Ear-notch samples are easy to collect, and preliminary studies have shown fresh samples to be stable, with no reduction in virus detection via ACE and RT-PCR in temperatures of  $-20^{\circ}\text{C}$ ,  $4^{\circ}\text{C}$ , and  $25^{\circ}\text{C}$  for 7 days (Ridpath JF, et al. Parameters of ear-notch samples for BVDV testing: stability, size requirements and viral load. 2006 AABP Conference, Minneapolis, MN Sept. 21–24). In 1 study,<sup>1</sup> ear-notch ACE was accurate in identifying 100% of PI animals detected by VI and RT-

PCR for the samples. However, in another study,<sup>4</sup> 88 of 21,743 cattle were initially positive by ear-notch ACE, but, on retesting, only 86 remained positive, indicating a need for repeat testing of animals to determine PI status.

Multiple studies have shown pooling to be a sensitive tool for detecting PI animals in a herd. For example, 1 viremic serum sample could be detected by RT-PCR when pooled with 100 serum samples,<sup>17</sup> and 1 PI was detected by RT-PCR from bulk milk in a lactating herd of 162.<sup>3</sup> Another study showed that RT-PCR had 100% accuracy in identifying 1 BVDV-positive animal in a pool of 100 by using ear-notch extractions.<sup>7</sup> Given the complicated situation of balancing accuracy and economics, information on how diagnostic laboratories deal with pooling is important, and a plan for pooling samples for BVDV testing should be developed. This survey indicates that a standard recommendation for pooling and an upper limit of samples for pooling does not exist in current diagnostic laboratory practice, because 46% of laboratories offered sample pooling, and there was no clear consensus on an upper limit for sample numbers that comprised the pool.

Current literature suggests that the appropriate procedure to detect PI animals involves performing more than 1 BVDV test over a 3- to 4-week period.<sup>1,8,14</sup> However, only 61% of diagnostic laboratories recommended retesting animals positive on 1 test. Only 55% of diagnostic laboratories surveyed were providing follow-up information to those producers with BVDV-positive tests during the testing period. Removing PI animals is the crux of a successful BVDV-control program and discussing the implications of positive test results with veterinarians and producers should be standard protocol. The discovery that only slightly more than half of laboratories provided follow-up information reveals a large opportunity to provide better education to producers.

The results of this survey indicated that simply by using clinical signs of BVDV for detecting BVDV in a herd may not be an effective means of BVDV control, because there was no significant difference between positive samples that were submitted because of clinical signs of BVDV versus submission for BVDV screening purposes. To achieve the goal of BVDV control and eradication, the most appropriate means of diagnostic testing is likely that of screening herds, a practice frequently used in other countries for BVDV control.

Sorting through the abundant and sometimes contradictory information regarding available BVDV diagnostic testing methods is a daunting task for diagnostic laboratories. If diagnostic laboratories do not provide a clear BVDV testing policy, it is unrealistic to expect that veterinarians and producers will have the time and the resources to determine the best testing strategy for their herds. In addition, this paper reports the number and types of tests performed but not the proficiency of the laboratories performing these tests. Because proficiency testing and validation documentation are not built into BVDV testing in the USA, little information is available to support producers in their choice of options. This is something the research and diagnostic communities need

to consider as we advance toward the goal of BVDV control and eventual eradication.

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## Appendix 1

### BVDV survey for diagnostic labs.

- Which state is your diagnostic lab located in?
- Please mark which of the following types of BVDV tests are available from your laboratory:
  - ☐ Virus Isolation
  - ☐ PCR on serum
  - ☐ PCR on buffy coat
  - ☐ PCR on milk
  - ☐ Ear notch IHC
  - ☐ Antigen ELISA on serum
  - ☐ Antigen ELISA on ear notch
  - ☐ Other Description:
- Please enter the number of BVDV tests your laboratory performed between **10/1/2004 and 10/1/2005** for each of the following test types. If you do not know how many of each test type was performed, please enter the total number of BVDV tests performed in your lab:
  - ☐ Virus Isolation
  - ☐ PCR on Serum
  - ☐ PCR on buffy coat
  - ☐ PCR on milk
  - ☐ Ear notch IHC
  - ☐ Antigen ELISA on serum
  - ☐ Antigen ELISA on ear notch
  - ☐ Other Description:
  - ☐ Total number of BVDV tests performed
- What was the number of tests found positive for BVDV in your laboratory between **10/1/2004 and 10/1/2005**?
- Did you request or recommend follow up retesting of positive samples?
  - ☐ Yes
  - ☐ No
  - ☐ Yes, depending on case. Explain:
- Did you supply those that submitted positive test samples with information on BVDV infections?
  - ☐ Yes
  - ☐ No

If yes, which form of information did you supply?

- ☐ Provided written information
- ☐ Provided oral information

7. Which of the following methods of testing does your lab utilize?

- ☐ We test individual samples
- ☐ We test pooled samples
- ☐ We test both individual and pooled samples

8. If you test **ONLY** individual samples, please skip to question 10. If you test pooled samples or test both individual and pooled samples:

Regarding the pooled BVDV samples, what is the upper limit for the number of individual samples that make up your pool to test?

9. If you test **ONLY** pooled samples, please skip to question 10. If you test both individual and pooled samples:

What percentage of your BVDV tests are performed on pooled samples? %

10. Please indicate (in a percentage) the frequency for each of the following reasons why BVDV testing is requested by submitters.

- % Requested because the clinical presentation suggested infection with BVDV
- % Requested because it is for a BVDV surveillance program

Upon completion of survey, please return to [edriskell@nadc.ars.usda.gov](mailto:edriskell@nadc.ars.usda.gov). Thank you.